

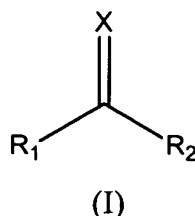
IN THE CLAIMS

Please amend the claims as follows:

Claims 1-34 (Cancelled).

35. (New) A method for increasing the sensitivity of a bio-luminescent assay comprising:
carrying out the assay in the presence of an organic compound that reduces luminescence that is not dependent on the presence of an analyte by at least about 10 fold and that reduces luminescence that is dependent on the presence of an analyte by less than about 7 fold,
wherein the organic compound has at least one carbon-sulfur bond (C-S), carbon-sulfur double bond (C=S), or carbon-selenium bond (C=Se).
36. (New) A method for increasing the sensitivity of a luminescent assay comprising:
carrying out the assay in the presence of an organic compound that reduces luminescence generated by luminogenic molecules not bound to an enzyme by at least about 10 fold and that reduces the luminescence generated by luminogenic molecules bound to an enzyme by less than about 7 fold,
wherein the organic compound has at least one carbon-sulfur bond (C-S), carbon-sulfur double bond (C=S), or carbon-selenium bond (C=Se).
37. (New) A method for increasing the sensitivity of a bio-luminescent assay comprising:
carrying out the assay in the presence of an organic compound that reduces autoluminescence by at least about 10 fold and that reduces luminescence that is dependent on the presence of an analyte by less than about 7 fold,
wherein the organic compound has at least one carbon-sulfur bond (C-S), carbon-sulfur double bond (C=S), or carbon-selenium bond (C=Se).
38. (New) The method of any one of claims 35, 36 and 37, wherein the organic compound is

a compound of formula (I):



wherein

X is S or Se;

R₁ and R₂ are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₁-C₂₀)alkoxy, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, heteroaryl, or NR_aR_b; or R₁ and R₂ taken together form a 5, 6, 7, or 8 membered saturated or unsaturated ring comprising carbon and optionally comprising 1, 2, or 3 heteroatoms selected from oxy (-O-), thio (-S-), or nitrogen (-NR_c-), wherein the ring is optionally substituted with 1, 2, or 3 halo, hydroxy, oxo, thioxo, carboxy, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₁-C₂₀)alkoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl; and

R_a, R_b and R_c are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, (C₂-C₂₀)alkynyl, aryl, heteroaryl;

wherein any (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₁-C₂₀)alkoxy, (C₂-C₂₀)alkenyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, or (C₂-C₂₀)alkynyl of R₁, R₂, R_a, R_b, and R_c is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more halo, hydroxy, mercapto, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo, or (C₁-C₂₀)alkoxycarbonyl; or a salt thereof.

39. (New) The method of any one of claims 35, 36 and 37, wherein the organic compound is a compound of formula R_3SH wherein

R_3 is (C_1-C_{20}) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_{20}) alkenyl, (C_2-C_{20}) alkynyl, aryl, or heteroaryl;

any (C_1-C_{20}) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_{20}) alkenyl, or (C_2-C_{20}) alkynyl of R_3 is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C_1-C_{20}) alkanoyl, (C_1-C_{20}) alkoxycarbonyl, aryl, heteroaryl, or NR_dR_e ;

R_d and R_e are each independently hydrogen, (C_1-C_{20}) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_{20}) alkenyl, (C_2-C_{20}) alkynyl, (C_1-C_{20}) alkanoyl, (C_1-C_{20}) alkoxycarbonyl, aryl, or heteroaryl; and

any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20}) alkanoyl, (C_1-C_{20}) alkanoyloxy, sulfo or (C_1-C_{20}) alkoxycarbonyl; or a salt thereof.

40. (New) The method of any one of claims 35, 36, and 37, wherein the organic compound is a compound of formula R_4NCS wherein

R_4 is (C_1-C_{20}) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_{20}) alkenyl, (C_2-C_{20}) alkynyl, aryl, or heteroaryl;

any (C_1-C_{20}) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_{20}) alkenyl, or (C_2-C_{20}) alkynyl of R_4 is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C_1-C_{20}) alkanoyl, (C_1-C_{20}) alkoxycarbonyl, aryl, heteroaryl, or NR_fR_g ;

R_f and R_g are each independently hydrogen, (C_1-C_{20}) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_{20}) alkenyl, (C_2-C_{20}) alkynyl, (C_1-C_{20}) alkanoyl, (C_1-C_{20}) alkoxycarbonyl, aryl, or heteroaryl; and

any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo, or (C₁-C₂₀)alkoxycarbonyl; or a salt thereof.

41. (New) The method of any one of claims 35, 36 and 37, wherein the organic compound is a compound of formula R₅-X-R₆ wherein

X is -S- or -Se-;

R₅ is (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl; and

R₆ is hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl;

or R₅ and R₆ taken together with X form a heteroaryl;

any (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, or (C₂-C₂₀)alkynyl of R₅ or R₆ is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, heteroaryl, or NR_kR_m;

R_k and R_m are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, or heteroaryl; and

any aryl or heteroaryl is optionally substituted with one or more halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo or (C₁-C₂₀)alkoxycarbonyl; or a salt thereof.

42. (New) The method of any one of claims 35, 36, and 37, wherein the organic compound is thiourea, selenourea, thioacetamide, 1-allyl-3-(2-hydroxy-ethyl)thiourea, 1-phenyl-3-thiazol-4-yl-thiourea, 5-methyl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one, (2-amino-thiazol-4-yl)-methoxyimino-acetic acid, 2-amino-ethanethiol, 1,5-dimercapto-pentane-2,4-diol, 2-amino-3-mercapto-propionic acid, or 2-acetyl-amino-3-mercapto-propionic acid .
43. (New) The method of any one of claims 35, 36, and 37, wherein the organic compound is thiourea, selenourea, 1-allyl-3-(2-hydroxy-ethyl)thiourea, or 5-methyl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one.
44. (New) The method of any one of claims 35, 36, and 37, wherein the luminescent assay employs a luciferase, aequorin, or obelin enzyme.
45. (New) The method of any one of claims 35, 36 and 37, wherein the luminescent assay employs firefly luciferase, *Renilla* luciferase, or *Cypridina* luciferase.
46. (New) The method of any one of claims 35, 36, and 37, wherein the organic compound is present in a concentration of from about 0.1 μ M to about 500 mM.
47. (New) The method of any one of claims 35, 36, and 37, wherein the assay is performed in the presence of whole cells.
48. (New) The method of any one of claims 35, 36, and 37, wherein the assay is carried out in a solvent comprising at least about 10% water by weight.

49. (New) The method of claim 35, wherein the luminescence that is dependent on the presence of an analyte is reduced by less than about 5 fold.

50. (New) The method of claim 36, wherein the luminescence generated by luminogenic molecules bound to an enzyme is reduced by less than about 5 fold.

51. (New) The method of claim 37, wherein the luminescence that is dependent on the presence of an analyte is reduced by less than about 5 fold.

52. (New) An assay kit comprising packaging material containing 1) a luminogenic substrate of a luminescent enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing luminescence that is not dependent on the presence of an analyte by at least about 10 fold and for reducing luminescence that is dependent on the presence of an analyte by less than about 7 fold, wherein the organic compound has at least one carbon-sulfur bond (C-S), carbon-sulfur double bond (C=S), or carbon-selenium bond (C=Se).

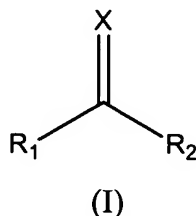
53. (New) An assay kit comprising packaging material containing 1) a luminogenic substrate of a luminescent enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing luminescence generated by luminogenic molecules not bound to an enzyme by at least about 10 fold and for reducing luminescence generated by luminogenic molecules bound to an enzyme by less than about 7 fold,

wherein the organic compound has at least one carbon-sulfur bond (C-S), carbon-sulfur double bond (C=S), or carbon-selenium bond (C=Se).

54. (New) An assay kit comprising packaging material containing 1) a luminogenic substrate of a luminescent enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing autoluminescence by at least about 10 fold, and for reducing luminescence that is dependent on the presence of an analyte by less than about 7 fold,

wherein the organic compound has at least one carbon-sulfur bond (C-S), carbon-sulfur double bond (C=S), or carbon-selenium bond (C=Se).

55. (New) The kit of any one of claims 52, 53, and 54, wherein the organic compound is a compound of formula (I):



wherein

X is S or Se;

R₁ and R₂ are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₁-C₂₀)alkoxy, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, heteroaryl, or NR_aR_b; or R₁ and R₂ taken together form a 5, 6, 7, or 8 membered saturated or unsaturated ring comprising carbon and optionally comprising 1, 2, or 3 heteroatoms selected from oxy (-O-), thio (-S-), or nitrogen (-NR_c-), wherein the ring is optionally substituted with 1, 2, or 3 halo, hydroxy, oxo, thioxo, carboxy, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₁-C₂₀)alkoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl; and

R_a, R_b and R_c are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, (C₂-C₂₀)alkynyl, aryl, heteroaryl;

wherein any (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₁-C₂₀)alkoxy, (C₂-C₂₀)alkenyl,

(C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, or (C₂-C₂₀)alkynyl of R₁, R₂, R_a, R_b, and R_c is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more halo, hydroxy, mercapto, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo, or (C₁-C₂₀)alkoxycarbonyl; or a salt thereof.

56. (New) The kit of any one of claims 52, 53, and 54, wherein the organic compound is a compound of formula R₃SH wherein

R₃ is (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl;

any (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, or (C₂-C₂₀)alkynyl of R₃ is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, heteroaryl, or NR_dR_e;

R_d and R_e are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, or heteroaryl; and

any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo or (C₁-C₂₀)alkoxycarbonyl; or a salt thereof.

57. (New) The kit of any one of claims 52, 53, and 54, wherein the organic compound is a compound of formula R₄NCS wherein

R₄ is (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl;

any (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, or (C₂-C₂₀)alkynyl of R₄ is

Serial No.: Unknown

Filed: Herewith

Title: METHOD FOR INCREASING LUMINESCENCE ASSAY SENSITIVITY

optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, heteroaryl, or NR_fR_g;

R_f and R_g are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, or heteroaryl; and

any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo, or (C₁-C₂₀)alkoxycarbonyl; or a salt thereof.

58. (New) The kit of any one of claims 52, 53 and 54, wherein the organic compound is a compound of formula R₅-X-R₆ wherein

X is -S- or -Se-;

R₅ is (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl; and

R₆ is hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, aryl, or heteroaryl;

or R₅ and R₆ taken together with X form a heteroaryl;

any (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, or (C₂-C₂₀)alkynyl of R₅ or R₆ is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, heteroaryl, or NR_kR_m;

R_k and R_m are each independently hydrogen, (C₁-C₂₀)alkyl, (C₃-C₈)cycloalkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkoxycarbonyl, aryl, or heteroaryl; and

any aryl or heteroaryl is optionally substituted with one or more halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C₁-C₂₀)alkanoyl, (C₁-C₂₀)alkanoyloxy, sulfo or (C₁-C₂₀)alkoxycarbonyl;
or a salt thereof.

59. (New) The kit of any one of claims 52, 53, and 54, wherein the enzyme substrate and the compound are each contained in a separate container.
60. (New) The kit of any one of claims 52, 53, and 54, wherein the enzyme substrate and the compound are contained in a single container.
61. (New) The kit of any one of claims 52, 53, and 54, further comprising a buffer solution suitable for use in a luminescent assay.
62. (New) The kit of claim 61, wherein the enzyme substrate and the buffer solution are contained in a single container.
63. (New) The kit of claim 61, wherein the compound and the buffer solution are contained in a single container.
64. (New) The kit of any one of claims 52, 53, and 54, further comprising a substrate for a second luminescent enzyme.
65. (New) The kit of any one of claims 52, 53, and 54, further comprising a quenching agent for a luminescent enzyme reaction.

PRELIMINARY AMENDMENT

Page 13

Serial No.: Unknown

Filed: Herewith

Title: METHOD FOR INCREASING LUMINESCENCE ASSAY SENSITIVITY

66. (New) The kit of any one of claims 52, 53, and 54, wherein the substrate is a substrate for firefly luciferase or *Renilla* luciferase.